September 11th, 2018

Submission of the revised manuscript: "Conducting Hyperscanning Experiments with Functional Near-Infrared Spectroscopy"

Dear Dr. Steindel,

We would like to thank you and both reviewers for the careful and thorough reading of this manuscript and for the thoughtful comments and constructive suggestions, which helped to improve its quality. Please find attached our point-by-point responses to each of the comments as well as the revised manuscript (with tracked changes).

Thank you for considering our manuscript for publication!

Kind regards,

Vanessa Reindl

Editorial comments:
Changes to be made by the Author(s) regarding the written manuscript:

1. **Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.**
2. **JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. You may use the generic term followed by "(see table of materials)" to draw the readers' attention to specific commercial names. Examples of commercial sounding language in your manuscript are: Easycap GmbH, Hitachi ETG-4000, Matlab, Wavelet Toolbox™, etc.**

   We have proofread the manuscript and removed all commercial names.

3. **Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).**

   We have now avoided all personal pronouns in the protocol text.

4. **Please revise the protocol to contain only action items that direct the reader to do something (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note." Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible.**

   We have revised the text as suggested and have used imperative tense throughout the protocol text (except for the "Notes").

5. **The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please simplify the Protocol so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step. Use sub-steps as necessary. Please move the discussion about the protocol to the Discussion.**

   Each step now contains a maximum of 4 sentences (mostly 2-3 sentences).

6. **Please add more details to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.**

   We have added more details to many of the protocol steps and added references where appropriate.

7. **1.1.2: How to attach the soft foam material? Is glue needed?**

We have added the information on how to attach the soft foam material to the protocol: "In order to make the caps more comfortable and minimize pressure marks, attach soft foam material (e.g., adhesive window sealing tape) at the inner side of the holder grid between the probe sockets and at the edges. Use double-faced adhesive tape if necessary." (step 1.1.4.)

8. **1.2.3: Please describe how this is actually done.**

   The step is now described in more detail:

   "1.2.2. Set all necessary options at the NIRS measurement system. Make sure that the device is set to event-related measurement and that the RS232 serial input, necessary for receiving triggers from the experimental paradigm, is active.

   NOTE: The experiment is an adapted version by a paradigm devised by Cui et al.[13], programmed in the non-commercial Psychophysics Toolbox extensions, version 3.0.11[17].

   1.2.3. Prepare the experimental paradigm by starting the technical computing software (see table of materials) that serves as base for the Psychophysics Toolbox extensions and setting the current directory to the folder that the paradigm is saved in."

9. **2.1.8: How to adjust the signal intensity?**

   It is now described how to adjust the signal intensity: "[…] by repeatedly clicking on the respective probe's symbol in the probe set monitor window of the NIRS measurement system." (step 2.1.10.)

10. **Step 3 and sub-steps: Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc.) to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.**

    We have added more details, such as button clicks, to the protocol, e.g. "Calculate oxy-Hb and deoxy-Hb concentration changes using the modified Beer-Lambert law by pressing the convert button in the main window. Enter the age of the subject and the distance between source and detector (e.g., 3 cm). Accept the default values for the molar absorption coefficients of oxy-Hb and deoxy-Hb at wavelength (λ) 1 and λ 2 as well as the default values for the differential pathlength factor (DPF) at λ 1 and λ 2" (step 3.1.2.)

    Moreover, we have included information about the analysis parameters in Figure 1.

11. **Lines 189-195: Please remove the weblinks and use a superscripted numbered reference instead.**

    We have removed the weblinks and replaced them with references.

12. **Discussion: Please also discuss any limitations of the technique.**

    We have added a new paragraph "Limitations" to the discussion:

"**Limitations.** Although fNIRS offers a promising, rapidly growing neuroimaging technique, some technical limitations associated with the device need to be considered when planning such a study (for a recent review see Pinti *et al.*[31]). In comparison to EEG and fMRI, fNIRS is more resistant to motion artifacts, yet, it still requires sufficient motion artifact control and detection. There are several potential causes of artifacts. First, some participants tend to move their head abruptly, in particular infants and children, and thereby might pull on the fiber tracts, affecting the optode contact. Developments of new fiberless devices are more robust to movement and even allow investigations of active tasks[31]. The use of a chin-rest can serve as additional motion artifact control, however, it limits the ability to record brain activities in natural interactions. Second, acquiring an adequate optode contact can be hindered by dark, curly and/ or thick hair of the participant. Placing the optodes can thus be time-consuming and a perfect signal is not always guaranteed. Third, depending on the fNIRS system, wearing optodes for a longer period of time can put pressure on the participant's head, which can be experienced as unpleasant. This does not only limit the recording time of the experiment but might also lead to more movement and artifacts (e.g., smaller children might pull on the cap). In addition to motion artifacts, it is noteworthy that fNIRS provides measures of the cortical surface only. Finally, there are no standardized data analysis guidelines yet. Several toolboxes were developed over the past years and first attempts were made to analyze the effectiveness of various preprocessing techniques (e.g., Brigadoi *et al.*[32] and Cooper *et al.*[33]). Moreover, the analytical protocol presented in this article shows one way to analyze fNIRS hyperscanning data. Importantly, the selected parameters of the analysis should be understood as one possible option and not as a standard guideline. Several other analytical protocols for fNIRS hyperscanning have been developed in the last years by different research groups (see for instance Cui *et al.*[13]; Hirsch *et al.*[34])."

13. References: Please do not abbreviate journal titles.

We have done accordingly.

14. Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file.

We have revised the table, as suggested.

Reviewers' comments:
Reviewer #1:

Manuscript Summary:
The manuscript describes a protocol on a functional near-infrared spectroscopy (fNIRS) hyperscanning experiment between dyads (e.g., parent - child, spouses, etc.) to assess brain-to-brain synchrony. The authors describe in detail the setup and preparation and also outline the relevant analyses steps.

Major Concerns:

1. **Preprocessing: This part is somehow confusing as it is not clear which steps are conducted by which program. For example, the ETG-4000 automatically calculates the concentration changes, it also has specific built-in low-/highpass filters, moving average and detrending procedures. It may be that I have difficulties as I do not know the SPM for NIRS toolbox, but it should be more clearly stated. Did you export the raw data without any filters? Such questions came to my mind when reading the part.**

   We have modified Figure 1 and included information on the data analysis steps, the specific analysis parameters as well as the toolboxes. Additionally, we have added further information in the text to clarify which steps were performed with which toolbox.

   We exported the raw data from the ETG-4000 without any filters. We have added this step to the protocol: "After the experiment, export the raw light intensity data as a text file by clicking on the text file out button and save the data. Do not apply any filters." (step 2.2.3.)

2. **What filters do you recommend and which parameters (e.g., frequency thresholds) should be used?**

   We have now included the parameters in Figure 1 (see above). We did not filter the data.

3. **Bad Channels: It would be advisable to present a figure on some of these artifacts. Are there any criteria for the percentage of CV allowed?**

   We have now included two examples of bad channels in Figure 1. The percentage of allowed CV differs between studies, often 10% is used. As an example for specific criteria, we have now referred the reader to the publication of van der Kant:

   "Calculate the coefficient of variation CV = SD/mean*100 for the raw attenuation data. Exclude channels in which the CV is above a predefined percentage (e.g., 10 %; see for instance van der Kant *et al* [22])." (step 3.2.2.).

4. **I am not in expert in wavelet analysis, so I do not know all the terms. Here, other reviewer should give comments.**
   Shortly, disadvantages of NIRS should be stated.

   We have included a new paragraph on the disadvantages of fNIRS in the discussion, focusing especially on practical issues (please see comment 12 to the editor).

5. **It could be mentioned which parameters to use further on. In the manuscript only one exemplary channel is shown with some coherence within the dyad.**

   In step 3.3.6. we describe the outcome measures used for statistical analysis. We have modified the step, which hopefully makes it more clear:

   "Calculate the percentage of significant WTC coefficients in the task-related frequency band for each task block in each channel and for each participant. Use this value as an outcome measure of brain-to-brain synchrony for further statistical analysis (for more information see Reindl et al.[11])."

   Minor Concerns:

6. **Abstract:**
   In the Abstract the abbreviation fNIRS should be introduced one sentence above when it is first mentioned.

   The abbreviation fNIRS is now introduced one sentence above.

7. **Introduction:**
   Studies 4 and 5 are not really hyperscanning studies, but studies on synchronization. Regarding the definition of Hyperscanning that is presented, this issue should be clarified. It also seems that none of these studies measured groups of participants simultaneously as you introduced before. There was a study by Dikker et al. (2017) in Current Biology which measured EEG in all children in a small classroom simultaneously. I am not sure if this is suited, but at least a group was hyperscanned. It is essential to measure simultaneously to get insights into social interaction or dependence processes (>1 brain) and not only social cognition, where one brain measure suffices.

   Thank you for the suggestion. We have changed it accordingly.

8. **It is true that NIRS has a much better temporal resolution than fMRI, but that is somehow put into perspective by the sluggishness of the BOLD signal. Beyond, developments of scanning protocols (multiband protocols) increase fMRI temporal resolution.**

   We have modified the sentence:
   "In addition, fNIRS data can be acquired at high sampling frequencies (e.g., 10 Hz), thus it highly oversamples the relatively slow hemodynamic response and thereby potentially provides a more complete temporal picture of the brain hemodynamics[10]." (lines 71 & 72)

9. **On the one hand, you state that fNIRS enables more realistic, ecologically valid setting, being an advantage compared to fMRI or EEG, on the other hand you write that you are using chin rests. Given this, it is more similar to fMRI and EEG.**

   We have now included the use of chin rests as a limitation in the discussion: "The use of a chin-rest can serve as additional motion artifact control, however, it limits the ability to record brain activities in natural interactions." (lines 480 & 481)

We agree that fNIRS is similar to EEG with respect to the experimental setting. However, we think that the experimental setting is more natural compared to fMRI hyperscanning, even when chin rests are used: “fNIRS is well suited for conducting hyperscanning experiments, especially with children, because it can be applied in less constrained and more natural settings than fMRI. Moreover, it is less prone to movement artifacts than both, fMRI and EEG[10].”

10. **I would not use the term pediatric as this implies patients.**

    We have now changed it to “children”.

11. **2.1.6 Will this be shown in the video? I only understand it since I know the ETG-4000.**

    Yes, this will be shown in the video.

12. **2.1.7 and 2.1.8 in both "yellow" channels are mentioned. Once you have to get the hair out of the way, once you should increase the signal.**

    First, the experimenter can try to brush the hair aside (step 2.1.9): “If a channel does not have a sufficient signal (i.e., if it is marked in yellow), gently put the hair underneath the surrounding probe tip aside. If necessary, push the probes further into their sockets but ensure the comfort of the participant. Check whether the signal quality has improved (i.e., the channel is now marked in green) by clicking on the auto gain button again.”

    If this is not successful, the experimenter can perform step 2.1.10: “If step 2.1.9. does not lead to a signal improvement, adjust the signal intensity. If there is too much signal (i.e., if the channel is marked in red), change the signal intensity to low signal intensity by repeatedly clicking on the respective probe’s symbol in the probe set monitor window of the NIRS measurement system. If there is not enough signal (i.e., if the channel is marked in yellow), change the signal intensity to high signal intensity, again by repeatedly clicking on the respective probe’s symbol”

13. **The figure legend should be more precise, for example by adding the details of processing, i.e. which parameters of preprocessing**

    Thank you for this suggestion. We have modified the figure legend and added the parameters in the figure:

    “Figure 1: Hyperscanning data analysis and representative results. Light intensity data is collected in 22 channels (CHs) of two participants. First, bad channels are detected and excluded from further analyses. Afterwards, light intensity data is converted to changes in oxy-hemoglobin (Δ Oxy-Hb) and deoxy-hemoglobin (Δ Deoxy-Hb). Signals are shown for one exemplary parent-child dyad in CH 8 during the cooperative condition. Data is preprocessed by reducing motion artifacts and slow drifts. Afterwards, the wavelet coherence is calculated from the preprocessed oxy-Hb signals. To estimate the significance of each wavelet coherence value, 300 surrogate time series are generated. If the observed wavelet coherence value is higher than 95% of the wavelet coherence values obtained from the surrogate time series at the same point in time and scale, it is regarded as significant. Significant wavelet coherence values are marked by solid lines surrounding the respective areas in the plot. Coherence in the task-related frequency band is depicted

within the black box. Please note that the analysis steps and the parametrization depicted in the figure should be understood as an example. The optimal parameterization depends on the data, e.g., different parameters of the MARA algorithm might work best for different types of artifacts[21], and there is no gold standard for any of the analysis steps yet."

Reviewers' comments:
Reviewer #2:

This interesting protocol, which was developed within the study of Reindl et al. (2018), described how to conduct fNIRS-based hyperscanning experiments and analyze interpersonal brain synchronization, and discussed several technical challenges and possible solutions. This protocol is novel and timely. The methods are sound. I believe that this protocol would be a nice addition to this field.

I just have a few minor concerns.

1. **line 75, it could be useful to also mention that this task was lately validated by Baker et al., 2016, Cheng et al., 2015, and Pan et al., 2017.**

   Baker, J. M., Liu, N., Cui, X., Vrticka, P., Saggar, M., Hosseini, S. H., & Reiss, A. L. (2016). Sex differences in neural and behavioral signatures of cooperation revealed by fNIRS hyperscanning. Scientific Reports, 6, 26492.

   Cheng, X., Li, X., & Hu, Y. (2015). Synchronous brain activity during cooperative exchange depends on gender of partner: A fNIRS-based hyperscanning study. Human Brain Mapping, 36(6), 2039-2048.

   Pan, Y., Cheng, X., Zhang, Z., Li, X., & Hu, Y. (2017). Cooperation in lovers: An fNIRS-based hyperscanning study. Human Brain Mapping, 38(2), 831-841.

   Thank you for this suggestion. We have added these studies.

2. **line 97 - line 103: Please mention that what your region of interest (ROI) was and why you chose it as the ROI.**

   We have now stated in the Introduction why we targeted prefrontal brain regions in the study: “Prefrontal brain regions were targeted as they had been identified as important regions for social interactive processes in previous hyperscanning studies[12].” (lines 81 - 83)

3. **line 232: WTC is the abbreviation of "wavelet transform coherence".**

   We have changed it accordingly.

4. **line 249: what is the meaning of "reasonable"?**

   We have changed the sentence to: “To examine the significance of the WTC coefficients and calculate their *p*-values, specify the number of surrogate time series (n>=300) and the ARMA model (e.g., AR (1)) in the AWCO function.” (step 3.3.3.)

5. **line 252: please simply explain how you chose the frequency of interest. These information (as well as the aforementioned region of interest) is helpful to readers.**

   We have now explained how to choose the frequency of interest: “Choose a frequency

band of interest in which the task-related brain-to-brain synchrony is expected to occur based on previous studies and visual inspection of the data (for an alternative approach see Nozawa *et al.*[26]).” (step 3.3.5.)

6. **I think it would be appropriate to add some reservations in the discussion. The current study did not provide the only way to analyze fNIRS hyperscanning data. It, however, provided a useful analytical protocol, which covered necessary and optional steps.**

   We absolutely agree that this is not the only possible way to analyze the data. We have added reservations to the protocol in the discussion:

   “Moreover, the analytical protocol presented in this article shows one possible way to analyze fNIRS hyperscanning data. Importantly, the selected parameters of the analysis should be understood as one possible option and not as a standard guideline. Several other analytical protocols for fNIRS hyperscanning have been developed in the last years by different research groups (see for instance Cui *et al.*[13]; Hirsch *et al.*[34]). (lines 491-495)

   Additionally, we have added some reservations to the figure legend:
   “Please note that the analysis steps and the parametrization depicted in the figure should be understood as an example. The optimal parameterization depends on the data, e.g., different parameters of the MARA algorithm might work best for different types of artifacts[21], and there is no gold standard for any of the analysis steps yet.” (lines 394 - 397)